Segmental allotetraploidy and allelic interactions in buffelgrass (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.) as revealed by genome mapping

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Abstract: Linkage analyses increasingly complement cytological and traditional plant breeding techniques by providing valuable information regarding genome organization and transmission genetics of complex polyploid species. This study reports a genome map of buffelgrass (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.). Maternal and paternal maps were constructed with restriction fragment length polymorphisms (RFLPs) segregating in 87 F_1 progeny from an intraspecific cross between two heterozygous genotypes. A survey of 862 heterologous cDNAs and gDNAs from across the Poaceae, as well as 443 buffelgrass cDNAs, yielded 100 and 360 polymorphic probes, respectively. The maternal map included 322 RFLPs, 47 linkage groups, and 3464 cM, whereas the paternal map contained 245 RFLPs, 42 linkage groups, and 2757 cM. Approximately 70 to 80% of the buffelgrass genome was covered, and the average marker spacing was 10.8 and 11.3 cM on the respective maps. Preferential pairing was indicated between many linkage groups, which supports cytological reports that buffelgrass is a segmental allotetraploid. More preferential pairing (disomy) was found in the maternal than paternal parent across linkage groups (55 vs. 38%) and loci (48 vs. 15%). Comparison of interval lengths in 15 allelic bridges indicated significantly less meiotic recombination in paternal gametes. Allelic interactions were detected in four regions of the maternal map and were absent in the paternal map.

Key words: linkage map, segmental allopolyploidy, restriction fragment length polymorphism, Poaceae, chromosome pairing.

Résumé : Les analyses de liaison génétique s'avèrent de plus en plus complémentaires aux techniques cytologiques et celles de l'amélioration génétique traditionnelle en fournissant des informations utiles concernant l'organisation du génome et l'hérédité chez des espèces polyploïdes complexes. Les auteurs rapportent ici une carte génomique du cenchrus cilié (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.). Des cartes, tant maternelle que paternelle, ont été produites à l'aide de marqueurs RFLP (polymorphisme de longueur des fragments de restriction) au sein d'une population en ségrégation comptant 87 individus F_1 dérivés d'un croisement interspécifique entre deux génotypes hétérozygotes. L'emploi de 862 clones d'ADNc ou d'ADNg hétérologues provenant de l'ensemble des hordées ainsi que 443 ADNc du cenchrus cilié a permis d'identifier 100 et 360 sondes polymorphes, respectivement. La carte maternelle comprenait 322 marqueurs RFLP formant 47 groupes de liaison et s'étendant sur 3464 cM. La carte paternelle comptait 245 marqueurs RFLP assemblés en 42 groupes de liaison et totalisait 2757 cM. Environ 70 à 80 % du génome du cenchrus cilié a ainsi été couvert et la distance moyenne entre les marqueurs était de 10,8 et 11,3 cM sur les cartes respectives. Des évidences d'appariement préférentiel entre plusieurs groupes de liaison ont été obtenues ce qui appuie des observations cytologiques antérieures à l'effet que le cenchrus cilié serait un allopolyploïde segmentaire. Davantage d'appariement préférentiel (disomie) a été observé chez le parent maternel que chez le parent paternel sur l'ensemble des groupes de liaison (55 contre 38 %) et des locus (48 comparativement à 15 %). Une comparaison de la longueur des intervalles chez 15 ponts alléliques a montré qu'il y avait significativement moins de recombinaison méiotique au sein des gamètes paternels. Des interactions alléliques ont été détectées dans quatre régions de la carte maternelle alors qu'elles étaient absentes de la carte paternelle.

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Mots clés : carte génétique, allopolyploïdie segmentaire, polymorphisme de longueur des fragments de restriction, hordées, appariement chromosomique.

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Introduction

Buffelgrass is an important perennial forage and range grass throughout the semi-arid tropics (Bogdan 1977). The species reproduces predominantly by aposporous apomixis, but sexual genotypes have been identified (Fisher et al. 1954; Snyder et al. 1955; Bashaw 1962; Bray 1978; Sherwood et al. 1980). Although the number of chromosomes have been reported to range from 32 to 54 for buffelgrass (Fisher et al. 1954; Hignight et al. 1991; Visser et al. 2000), most genotypes are tetraploids ($2n = 4x = 36$). Cytological observations have indicated that the 36-chromosome types are segmental allotetraploids with 10–14 bivalents and 2–4 quadrivalents at diakinesis (Fisher et al. 1954; Snyder et al. 1955). Even though spindle formation and cytokinesis appear normal, 1–5 lagging univalents are occasionally observed at anaphase I (Fisher et al. 1954; Snyder et al. 1955; Hignight et al. 1991). Thus, disomic, tetrasomic, and distorted inheritances are possible. Segregation studies of apomixis in buffelgrass have reported both disomic (Taliaferro and Bashaw 1966) and tetrasomic (Sherwood et al. 1994) inheritances, with potential for recessive lethality.

Linkage analyses in polyploid species of uncertain genome constitution have been used to distinguish auto- from allopolyploidy, homology from homoeology, and disomy from tetrasomy (Ritter et al. 1990; Wu et al. 1992). Differences in recombination levels can be detected by comparing homologous segments within parental maps (Wang et al. 1995; Kearsey et al. 1996; Korzun et al. 1996; Havekes et al. 1997). Genomic rearrangements relative to polyploid formation can be revealed, in general, by RFLP characterization (Chittenden et al. 1994), and specifically, by comparative mapping (Ming et al. 1998).

Wu et al. (1992) proposed using simplex markers, single-dose restriction fragments (SDRFs), to develop genetic maps in heterozygous polyploids. This method has been used to study complex genomes in *Saccharum spontaneum* L., *Saccharum* interspecific hybrids, and cassava (*Manihot esculenta* Crantz). For example, *S. spontaneum* ($2n = 8x = 64$) was determined to be an autopolyploid (Al-Janabi et al. 1993; Da Silva et al. 1993); the frequency of preferential chromosome pairing was quantified in *Saccharum* interspecific hybrids (Ming et al. 1998); and cassava appeared to be a segmental allopolyploid (Fregene et al. 1997).

The use of SDRFs, which segregate at 1:1, excludes the identification of genomic regions with non-Mendelian segregation. However, SDRFs may be used to detect transmission ratio distortion between allelic combinations. Two alleles (A and B) for genes on independent chromosomes are expected to segregate 1:1:1:1 (A/B, A/–, –/B, and –/–) during meiosis. Likewise, SDRFs that map to separate linkage groups should segregate in the same manner. Deviations from the expected ratio would indicate that one of the allelic alternatives is transmitted to the progeny at higher frequencies. Physiological and (or) environmental factors may be involved, but evidence of allelic interactions has been reported in different species (Maguire 1963; Rick 1966; Sano 1990; Manabe et al. 1999).

A buffelgrass linkage map will contribute to comparative studies of genome-wide maps in other forage grasses (Liu et al. 1994; Xu et al. 1995; Hayward et al. 1998; Ortiz et al. 2001; Porceddu et al. 2002), and to mapping studies of apomixis in *Tripsacum* (Leblanc et al. 1995; Kindiger et al. 1996; Grimanelli et al. 1998; Blakey et al. 2001), *Pennisetum* (Ozias-Akins et al. 1993, 1998; Gustine et al. 1997; Jessup et al. 2002), *Brachiaria* (Pessino et al. 1997, 1998), and *Poa* (Albertini et al. 2001). The objective of this study was to better understand transmission genetics in buffelgrass and provide useful DNA markers for grass breeding programs through the construction of a framework buffelgrass linkage map.

Materials and methods

Plant material

A full-sib buffelgrass population, derived from crossing a highly heterozygous, sexual genotype (90C48507) with a highly heterozygous, apomictic genotype (PI 409164), was the source of 87 F_1 hybrids included in the mapping population (Jessup et al. 2002). Because PI 409164 was used as its grandparent, 90C48507 possessed an inbreeding coefficient of $F = 0.25$. While this means there may be some chromosomal segments lacking DNA polymorphism, the parents differed for numerous traits of importance to future mapping studies: inflorescence type (birdwoodgrass vs. buffelgrass), rhizomes (absence vs. presence), method of reproduction (sexual vs. apomictic), plant height (short vs. tall), plant color (green vs. blue), flowering time (early vs. late), and percentage of flowering tillers (high vs. low).

Analysis of molecular markers

Genomic DNA extraction was adapted from the protocol of Causse et al. (1994). Ten micrograms of buffelgrass genomic DNA was digested with *Eco*RI, *Hin*dIII, or *Xba*I, according to the manufacturer's instructions. Southern blotting, radioactive labeling, and autoradiography were as described by Chittenden et al. (1994).

A cDNA library constructed from pistils of an obligate apomictic buffelgrass plant selected from the full-sib population was developed using the Stratagene (La Jolla, Calif.) ZAP-cDNA Synthesis Kit. Bacterial clones from the library were obtained by en masse phagemid excision, followed by two cycles of selection for recombinant clones on ampicillin plates containing X-Gal and isopropylthio-β-D-galactoside (IPTG) (Sambrook et al. 1989). Inserts were amplified by PCR from bacterial lysate (McCabe 1990), and an aliquot of the products was electrophoresed in 1% w/v agarose. Clones that gave multiple products were discarded. Sephadex G50 (Sigma St. Louis, Mo.) spun mini-columns were used to sep-

arate PCR products from excess reaction components (Sambrook et al. 1989). Dot blots with 20 ng of DNA from each probe were hybridized with leaf cDNA to identify and eliminate repetitive elements. A total of 433 suitable probes were designated "pPAP", for plasmid *Pennisetum* apomictic pistil. Ten previously isolated buffelgrass differential display products (*Pennisetum ciliare* apomictic (Pca) and *Pennisetum ciliare* sexual (Pcs)) (Vielle-Calzada et al. 1996) were also analyzed.

In addition, heterologous DNA probes from several sources were surveyed: 21 barley (*Hordeum vulgare* L. subsp. *vulgare*) cDNAs (BCD), 32 bermudagrass (*Cynodon dactylon* (L.) Pers.) gDNAs (pCD), 37 johnsongrass (*Sorghum halepense* (L.) Pers.) differential display products (pSHR), 250 maize (*Zea mays* L. subsp. *mays*) cDNAs (CSU), 28 maize gDNAs (BNL, UMC), 49 pearl millet (*Pennisetum glaucum* (L.) R. Br.) gDNAs (M), 105 rice (*Oryza sativa* L.) cDNAs (C, RZ), 88 rice gDNAs (G, L, RG, V, Y), 29 sorghum (*Sorghum bicolor* (L.) Moench) cDNAs (HHU), 148 sorghum gDNAs (pSB, SHO), 29 sugarcane (*Saccharum officinarum* L.) cDNAs (CDSB, CDSC, CDSR), and 46 oat (*Avena sativa* L.) cDNAs (CDO). All gDNAs were hypomethylated (*Pst*I digested). Probes were generously provided by A. Paterson (University of Georgia) (pCD, pSHR, pSB, SHO); S. Tanksley, M. Sorrells, and S. McCouch (Cornell University, Ithaca, N.Y.) (BCD, RZ, RG, CDO); P. Moore (John Innes Centre, Norwich, U.K.) (CDSB, CDSC, CDSR); T. Sasaki and Y. Nagamura (National Institute of Agrobacterial Sciences, Japan) (G, L, V, Y); P. Westhoff (USDA–ARS) (HHU); and M. Gale (John Innes Centre) (M).

Surveyed homologous probes were further classified as low copy (≤4 bands), multiple copy (>4 bands), or repetitive (smear). Polymorphism was noted for each restriction enzyme in low copy probes for buffelgrass survey blots and used to infer the type of genomic rearrangements (insertions–deletions (indels) versus base substitutions) contributing to polymorphism between the parents. Only low-copy DNA probes that yielded unambiguous results for all three restriction enzymes were included in this analysis.

Hybridity of all individuals used in the mapping population was verified through the use of 3 to 4 dose restriction fragments, which transmit at least one copy to all progeny. Markers of this type derived from pollen of the paternal parent, which were absent in the female parent, were considered. Any progeny lacking such bands, either through an undesired outcross or self-fertilization, would be removed from the mapping population.

Linkage analysis

Probes revealing polymorphism on buffelgrass survey blots were hybridized to the mapping filters. Because the parental buffelgrass lines are highly divergent polyploids, each polymorphic band was treated as a locus with dominant gene action. Individual bands present in one parent and absent in the other parent were scored for presence or absence in the progeny. A χ^2 test was used to identify SDRFs by their 1:1 segregation ratio at a significance level of 1% (Wu et al. 1992). A nonsignificant test indicated that a given band was an SDRF and could be considered in the linkage analysis.

Because an SDRF only reveals segregation in the gametes of one parent, an RFLP map of each parent's respective SDRFs was constructed using Mapmaker 3.0 (Lander et al. 1987). SDRFs were treated as backcross data. Based on the use of 87 individuals, an LOD score of 4.0 and recombination fraction of 0.30 were set as the linkage thresholds. The maximum detectable recombination fraction (max_r) in the buffelgrass mapping population is 0.435 at a 98% confidence level for linkage in the coupling and repulsion phases of an allotetraploid, as well as in coupling-phase linkage of an autotetraploid (Wu et al. 1992). However, max_r would only be 0.126 for repulsion-phase linkage of an autotetraploid. The use of $max_r = 0.30$ was, therefore, a conservative estimate for our population in all but the last case. Map units, in centimorgans (cM), were derived using the Kosambi (1944) function. Maximum-likelihood orders of markers were verified using the "ripple" function, with those at LOD ≥ 2.0 being placed on the framework map and all others added at the most likely interval between framework markers.

Genome length, *G*, was estimated from partial linkage data according to the equation of Hulbert et al. (1988):

$$G = MX/K$$

where *M* is the number of informative meioses, *X* is an interval (cM) at some minimum LOD score, and *K* is the actual number of pairs of markers found to border the interval X or less. A maximum value for *M* equals the number of pairwise combinations for linked markers (*N*), or $N(N - 1)/2$. The proportion of genome coverage (*C*) in terms of the probability (*P*) of a random point not being covered was calculated as suggested by Bishop et al. (1983). The equation used was as follows:

$$C = 1 - (2r)/(n + 1)[(1 - x/2t)^{n+1} - (1 - x/t)^{n+1}] + (1 - rx/t)(1 - x/t)^n,$$

where *r* is the number of linkage groups, *x* is an interval (cM), *n* is the number of intervals, and *t* is the sum of linkage group lengths (cM).

An evaluation of preferential pairing between chromosomes was accomplished by comparing the ratio of repulsion- versus coupling-phase linkages. To detect repulsion-phase linkages, two-point linkage analyses were repeated with the allele states inverted. Using a χ^2 test, a 1:1 ratio would indicate allotetraploidy (disomic inheritance). Two linkage groups with markers in repulsion along their length would be interpreted as pairing partners (homologous chromosomes). A 0.25:1 ratio would indicate autotetraploidy (tetrasomic inheritance). Any intermediate ratio would suggest partial preferential or multivalent chromosomal pairing and a more complex form of polyploidy (Wu et al. 1992).

Allelic bridges were used to identify and orient analogous linkage groups in the paternal and maternal maps (Ritter et al. 1991). Such probes detect an SDRF unique to each parent and a fragment common between both parents. A paired *t* test of male and female map intervals from homologous regions (as determined by allelic bridges) was conducted to compare recombination rates in the parents.

Segregation distortion was analyzed between each mapped SDRF and all other mapped SDRFs, excluding those on the same linkage group and its homolog. Deviations from the

Table 1. Restriction enzymes revealing polymorphism with low-copy pPAP probes in *Pennisetum ciliare*.

	None	Any one restriction enzyme	Any two restriction enzymes	All three restriction enzymes
Observed	37	101	52	75
Expected	25	90	108	42

Note: $\chi^2 = 113.26$, $P \leq 0.0001$.

expected 1:1:1:1 were considered significant at LOD ≥ 4.0. Analyses of each class would identify which allelic combination occurred in excess.

Results

Assessment of DNA polymorphism

Forty-nine uncharacterized repetitive elements were eliminated because they could not be scored on mapping filters. A survey of 862 heterologous cDNAs and gDNAs from across the Poaceae and 443 buffelgrass cDNAs yielded 100 and 360 polymorphic probes, respectively. For the three restriction enzymes that were used, the parents were polymorphic for 90% of the low-copy pPAP probes: 57% with *Eco*RI, 57% with *Hin*dIII, and 49% with *Xba*I.

Low-copy probes polymorphic with zero, one, or all three of the enzymes occurred more frequently than expected, whereas probes polymorphic with two of the enzymes occurred less frequently than expected (Table 1). RFLPs from localized genomic rearrangements (indels) increased the probability that a probe would detect polymorphism with either zero or all three of the restriction enzymes. RFLPs from base substitutions within restriction sites caused polymorphisms for single enzymes to be independent of one another, and the occurrence of "two-" and "three-enzyme" probes were predictable from the polymorphism rates of each single enzyme.

Segregation analysis

The 460 polymorphic probes were scored in 87 F_1 hybrids, yielding segregation data for 400 SDRFs in the female parent and 298 SDRFs in the male parent. A total of 19 probes identified 3 to 4 dose restriction fragments that were present in the paternal parent and absent in the maternal parent. All of these were present in 86 of the F_1 hybrids but absent in one hybrid. This plant (92BWB-77) was either a maternal self or an outcross, and its removal resulted in a final mapping population of 86 individuals.

Linkage analysis

Linkage analyses of all SDRFs were conducted using MAPMAKER 3.0. The maternal map included 322 SDRFs in 47 linkage groups, which spanned 3464 cM and had an average interval between markers of 10.8 cM. The paternal map included 245 SDRFs in 42 linkage groups with 2757 cM and an average interval between markers of 11.3 cM. Both maps are shown in Fig. 1. Seventy-eight and 53 markers remained unlinked in the maternal and paternal maps, respectively. Analysis of linkage data gave estimates for overall genome length (4309 vs. 3679 cM) and coverage (74% vs. 70%) in the maternal and paternal maps, respectively. Final estimates of genome coverage (80% for the maternal map and 75% for the paternal map) were obtained by dividing the map lengths into the estimated genome lengths.

Chromosome associations

The number of chromosomes counted in root tip cells confirmed that both parents had 36 chromosomes. Loci linked in repulsion-phase were compared with loci linked in coupling-phase within each linkage group to determine the frequency of preferential or random chromosome assortment (Table 2). Repulsion: coupling-phase SDRF ratios of 1:1 predict disomy, while 0.25:1 ratios predict tetrasomy (Wu et al. 1992). Disomy was most common in the female parent, but disomy and tetrasomy occurred at similar frequencies in the male parent. The detection of repulsion-phase associations within 26 linkage groups in the maternal map supports cytological evidence of 10 to 14 bivalents during meiosis in buffelgrass (Fisher et al. 1954). Repulsion was detected in only 16 linkage groups of the paternal map; however, the presence of two unlinked markers in repulsion suggests that more repulsion-phase associations exist.

Repulsion was observed for 48% of the probes in the maternal map, compared with 15% of probes in the paternal map. Assuming random chromosome assortment, diploids and allopolyploids would have repulsion at 50% of their loci, whereas a segmental allopolyploid with 14 bivalents and two quadrivalents should have repulsion at 44% of its loci.

Duplication of RFLP loci

Of the 460 polymorphic probes, 369 (80%) revealed bands segregating for multiple loci. Duplicate loci and repulsion data were used when multiple linkage groups shared common markers to identify putative homologous and homoeologous relationships. Of these duplicated loci, only 21 (5.7%) were classified as allelic bridges. Allelic bridges have a SDRF unique to each parent, a fragment common to both parents, and analogous positions in linkage groups of the maternal and paternal maps (Ritter et al. 1991). A total of 26 and 28 loci in the maternal and paternal maps, respectively, made up 15 allelic bridges (Fig. 1). Ten allelic bridge intervals were larger in the maternal map than in the paternal map. Five allelic bridge intervals were larger in the paternal map than in the maternal map. Average bridge lengths in the female map were significantly greater ($P < 0.03$), suggesting differences in recombination rates between the parents. Comparison of the overall map lengths was not considered because of the unequal number of markers in the maps, as well as the a priori knowledge of inbreeding ($F = 0.25$) in the maternal parent.

Segregation distortion

Transmission ratio distortion, though undetected in the paternal map, was observed between markers on two pairs of

Fig. 1. Maternal (white linkage groups) and paternal (black linkage groups) RFLP maps of *Pennisetum ciliare*. Framework map (LOD > 2.0) markers and map distances (Kosambi map units) are shown to the right and left of the horizontal lines, respectively. Probes are denoted as follows: buffelgrass cDNAs (pPAP as "P", Pca, Pcs), sorghum cDNAs (HHU), sorghum gDNAs (pSB, SHO), barley cDNAs (BCD), bermudagrass gDNAs (pCD), johnsongrass cDNAs (pSHR), maize cDNAs (CSU), maize gDNAs (BNL, UMC), pearl millet gDNAs (M), rice cDNAs (C, RZ), rice gDNAs (G, L, RG, V, Y), sugarcane cDNAs (CDSB, CDSC, CDSR), and oat cDNAs (CDO). Markers followed by a, b, c, etc., correspond to probes detecting polymorphisms at multiple loci on the maps. Allelic bridge loci between the maps are shown in bold, and markers with segregation distortion are italicized in boxes. Linkage group names ending with a or b indicate homologous (disomic) chromosome pairs. Bold, curved lines represent loose linkages (recombination fraction > 0.30).

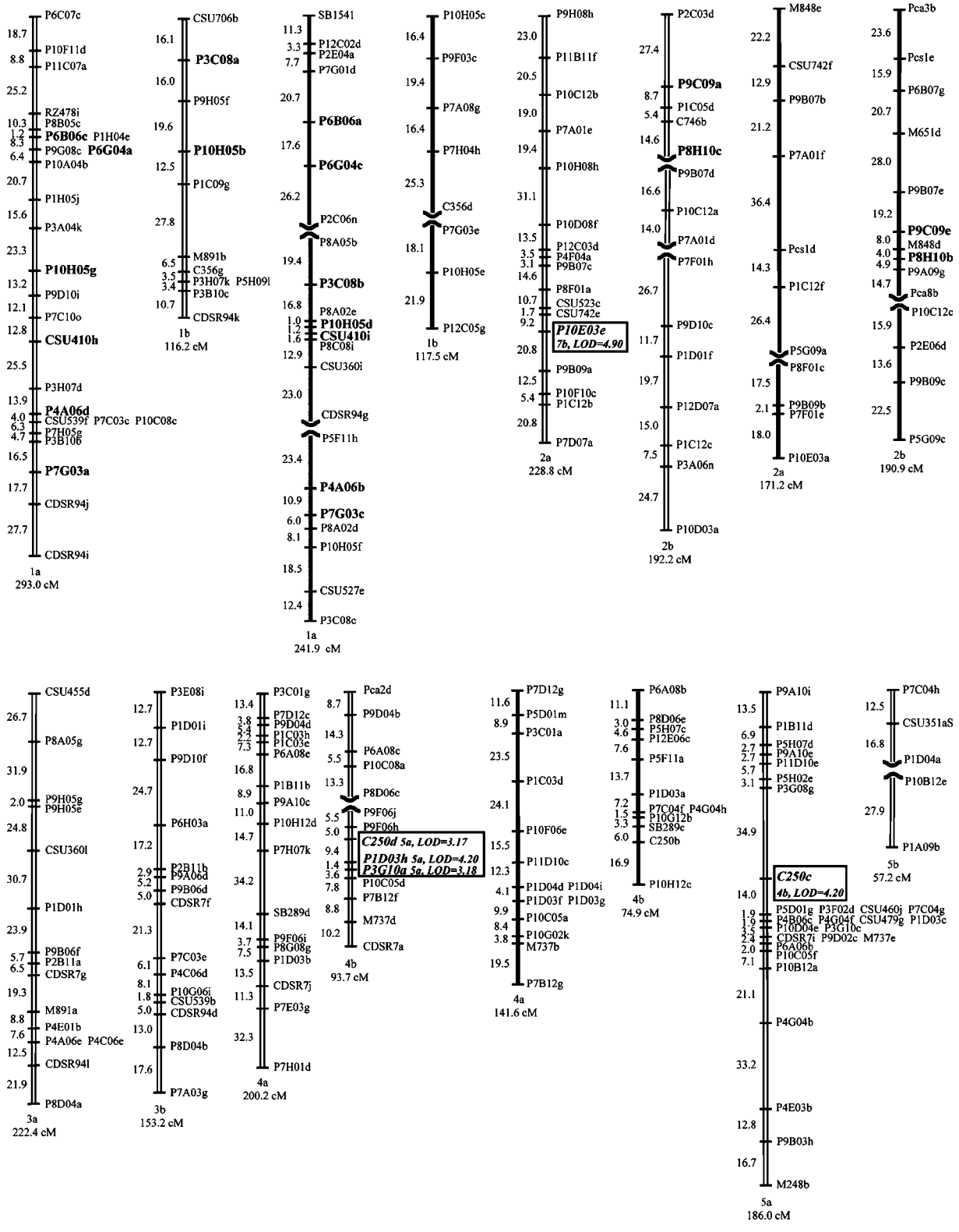
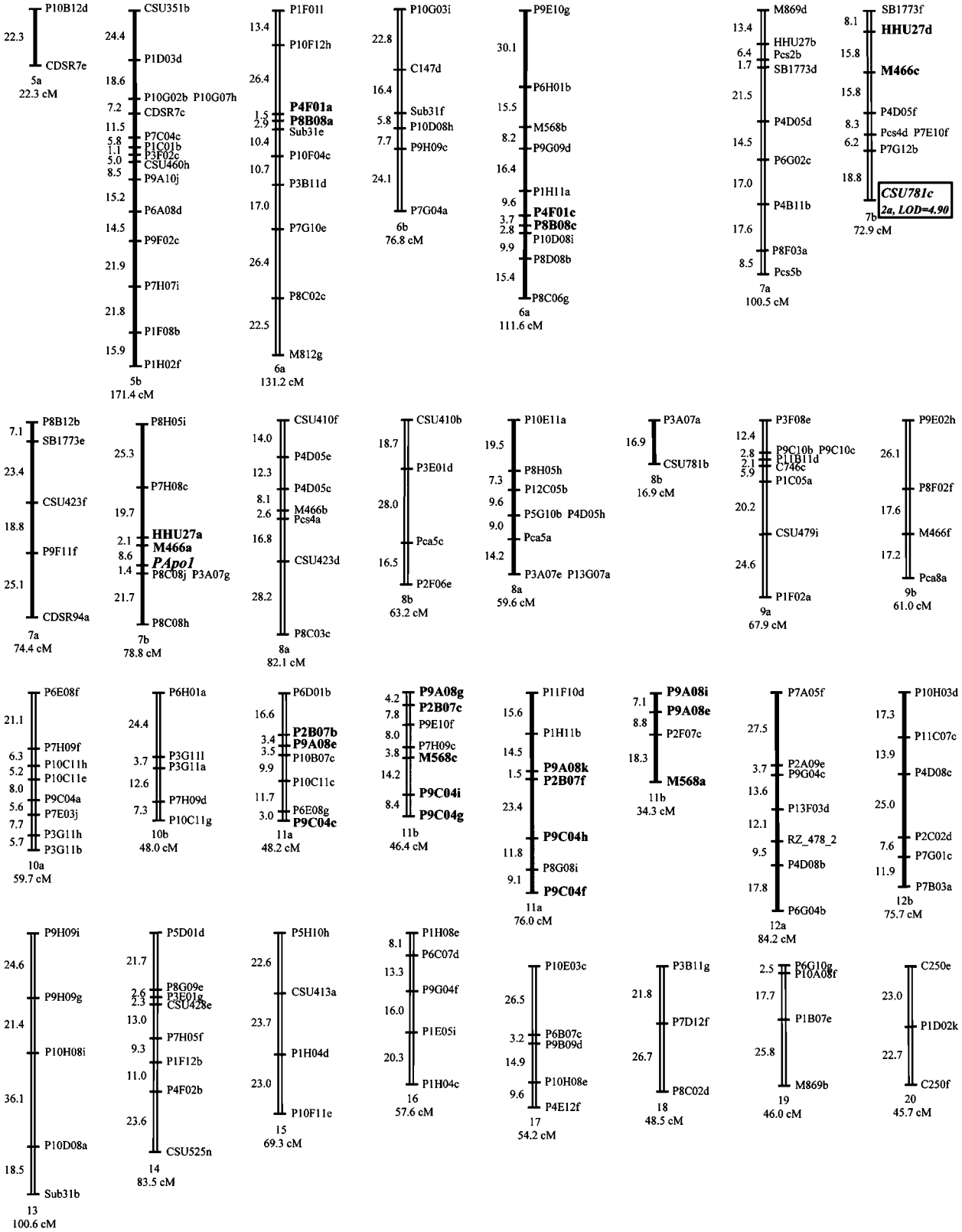
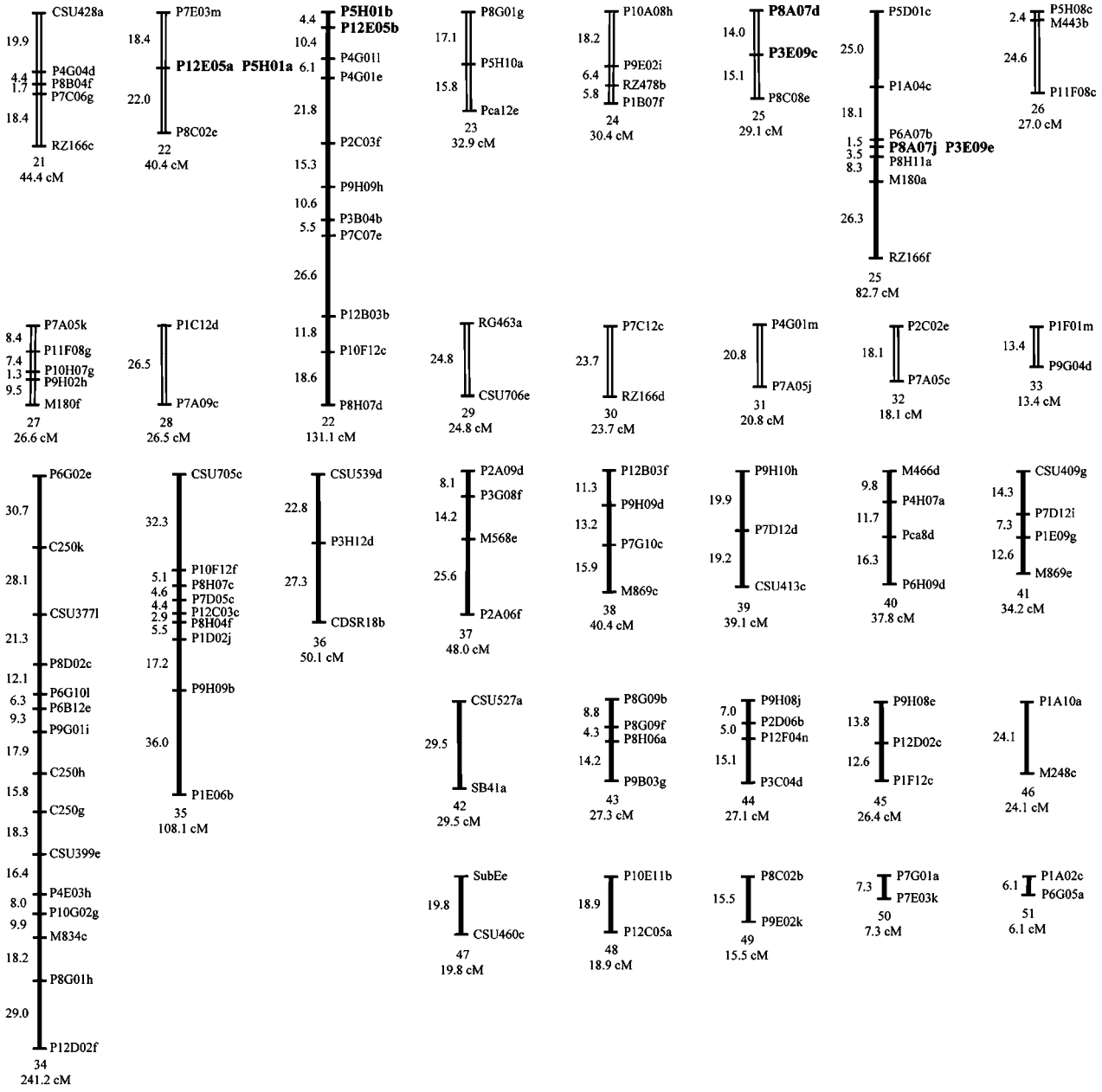


Fig. 1 (*continued*).



linkage groups in the maternal map (Fig. 1). The first pair consisted of markers P10E03e on linkage group 2a and CSU781c on linkage group 7b. The second pair consisted of markers P1D03h on linkage group 4b and C250c on linkage group 5a. The markers on linkage group 4b gave results expected of a region with segregation distortion, because flanking markers C250d and P3G10a were distorted to a lesser extent with C250c. The allelic heterozygotes P10E03e/– and

Fig. 1 (*concluded*).



P1D03h/– were transmitted in excess to the progeny (Table 3). In both cases, the A/B and –/– homozygote classes were transmitted at a frequency less than expected.

Discussion

The prevalence of cDNA markers (80%) identifying duplicated loci confirmed that buffelgrass is a polyploid species containing related, but divergent, genomes. Because only 5.7% of the duplicated loci had homologous map positions in the parental maps, numerous genome organization differences exist between these genotypes. The correlation of polymorphism frequencies across restriction enzymes further suggests that localized genome rearrangements (indels) account for many RFLPs in buffelgrass. The lost, gained, and novel fragments resulting from these events may help explain the high levels of heterozygosity in buffelgrass. Such genome changes are important subsequent to polyploid formation (Song et al. 1995) and occur at least as frequently as inter-chromosomal or long-distance transpositions (Seoighe et al. 2000). However, a high frequency of such rearrangements could complicate comparative mapping between buffelgrass and established linkage maps.

An inbreeding coefficient of $F = 0.25$ was calculated for the maternal parent based on parentage. However, estimates of genome coverage (74–80%), the presence of regions with segregation distortion, and the remaining unlinked SDRFs indicate that additional regions of the buffelgrass genome

Table 2. Repulsion- vs. coupling-phase linkage ratios across linkage groups in the maternal and paternal maps.

	1:1 (disomy)	0.25:1 (tetrasomy)	0.25–1:1 (complex)
Maternal parent (90C48507)	19	2	5
Paternal parent (PI409164)	6	5	5
Expected (14 IIs, 2 IVs)	28	8	0
Expected (12 IIs, 3 IVs)	24	12	0

Note: IIs, bivalents; IVs, quadravalents.

Table 3. Transmission distortion of allelic combinations in the maternal map.

	A/B	A/–	–/B	–/–
Expected (n = 86)	21.5	21.5	21.5	21.5
Observed				
P10E03e/CSU781c (χ^2 = 28.33, $P \leq 0.0001$)	8	40	25	13
P1D03h/C250c (χ^2 = 22.56, $P \leq 0.0001$)	8	37	26	15

can be mapped. Given these results, it is likely that the actual amount of inbreeding in the maternal parent was less than predicted.

The presence of repulsion within most linkage groups implies that significant preferential chromosome pairing occurs in buffelgrass. This supports cytological evidence of segmental allotetraploidy in the species (Fisher et al. 1954: Snyder et al. 1955). However, the large difference in repulsion-phase associations across loci in the maternal and paternal maps (48 vs. 15%) suggests that chromosome behavior varies between female and male gametes (Fogwill 1958; Mogensen 1977; Ross et al. 1996; Havekes et al. 1997). In comparison to this three-fold difference, repulsion across linkage groups in the maternal and paternal maps (55% vs. 38%) was minor. One possible explanation for these results is that the female parent may have higher crossing-over frequencies along the length of its paired chromosomes. With the general acceptance that chromosome "alignment" and "synapsis" are different processes (Loidl 1990; Kleckner 1996; Cook 1997; Moore 2000), similar preferential pairing and dissimilar chiasma formation between genotypes is also plausible. Alternatively, a shorter meiotic cycle (Havekes et al. 1997) or greater chromatin modifications in gametes of the male parent are possible.

Allelic bridges aligned several linkage groups between the parents. This suggests either a genome-wide reduction in recombination of gametes segregating from the male parent or an increase in recombinant gametes from the female parent. Although not well understood, this phenomenon has been reported in other species (Vizir and Korol 1990: de Vincente and Tanksley 1991; Wang et al. 1995; Kearsey et al. 1996; Korzun et al. 1996; Fregene et al. 1997). Because repulsion levels in the female parent approached those expected across linkage groups and loci, suppressed recombination in the male parent's gametes is more likely than increased recombination in the female parent's gametes. It also suggests that cytoplasmic–nuclear interactions, which have been implicated in nuclear genome changes of polyploids (Soltis and Soltis 1995) and chiasma formation (Kruleva et al. 1992), may occur in buffelgrass.

Previous SDRF-based linkage studies have not mapped regions of segregation distortion (Al-Janabi et al. 1993; Da Silva et al. 1993; Fregene et al. 1997; Ming et al. 1998). However, genetic analyses of such regions provide information useful to breeding programs. Molecular markers in affected regions can be used to select and increase the frequency of a favored heterozygote class.

The transmission ratio distortion observed in buffelgrass occurred only through the maternal parent. This behavior is similar to that reported in maize (Maguire 1963), wheat (Scoles and Kibirge-Sebunya 1983; Manabe et al. 1999), and barley (Konishi and Fukushima 1992). Because the four SDRFs (cDNAs) involved in these allelic interactions were mapped in both parents, genome rearrangements in these regions were identified between the parents. For the allelic interaction involving the P10E03/CSU781 loci, P10E03 mapped to aligned homologs (2a) in both parental maps. In contrast, CSU 781 mapped to 7b in the maternal parent and to a homeolog of 7b (8b) in the paternal parent. Thus, a translocation may have occurred in this region. For the interaction between the P1D03/C250 loci, P1D03 mapped to aligned homologs (4b) in both parental maps; however, C250 mapped to 5a in the maternal map and to three loci on an independent linkage group (34) in the paternal map. In this case, both translocation and gene duplication may have occurred in this region.

Two additional factors warrant further consideration concerning the parental differences in chromosome associations and allelic interactions found in this study. First, the parents differed in method of reproduction. The molecular mechanism of apomixis remains unknown and may involve components of meiotic regulatory pathways. This is especially noteworthy, because linkage group 7b contains the locus for apospory (PApo1) in buffelgrass. Second, the maternal (sexual) parent is of recent origin and was derived from crosses between several diverse genotypes (Jessup et al. 2002). In contrast, the paternal (apomictic) parent is a plant introduction that is probably of much earlier origin. According to the model proposed by Stebbins (1950), the maternal parent may be an "unstable" segmental polyploid and subject to chromosome alterations through selection pressures. In contrast, the paternal parent may be a "stable" segmental polyploid as a result of either (*i*) sufficient time for the removal of unfavorable genetic elements, or (*ii*) escape from

selection pressures through apomictic reproduction. Linkage analyses of multiple genotypes in genomic regions of interest will help resolve these possibilities; however, the recent origin of all identified sexual buffelgrass genotypes may require the use of other methods.

With 90% of the low-copy DNA clones mapped and 70–80% of the buffelgrass genome covered, the hybrids used in this study are well suited for linkage analyses. Placing more markers on the maps will increase resolution and genome coverage. The recent expansion of our mapping population to more than 200 individuals will facilitate the identification of QTLs for traits of agronomic interest. The use of heterologous probes from across the *Poaceae* will allow extensive comparative mapping with the buffelgrass linkage map. Information gained can be used to increase our understanding of genome organization and polyploid evolution, as well as to provide molecular markers that will be useful to grass breeding programs.

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